

REMARKS

Claims 1-14 are pending in this application.

I. Oath

A copy of the original oath filed with the reissue application is being submitted at the Examiner's request.

II. Claim Rejection under 35 U.S.C. § 103(a)

Claims 1-14 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Fenn et al '84 and Dolan et al. '88. Applicant respectfully disagrees because not all claim limitations of claims 1-14 are taught or suggested by the cited references or are within the knowledge of one of ordinary skills in the art at the time of the present invention.

The Examiner has acknowledged that the compositions disclosed in either Fenn or Dolan are much more dilute than the compositions of the present application. The Examiner cited Figures 1 and 5 of Fenn and Figures 1-3 of Dolan and reasoned that because the dose/response curves in Fenn and Dolan showed enhanced efficacy, one of ordinary skills would be motivated to increase the concentration of PO₃ or PO₄ in the composition. However, none of the dose/response curves in Figures 1 and 5 of Fenn and Figures 1-3 of Dolan show a combined first salt of PO₃ and second salt of PO₄ as instantly claimed. Even if one would be motivated to extrapolate from the curves of Figures 1 and 5 of Fenn and Figures 1-3 of Dolan, one would only arrive at a composition with a higher concentration of PO₃, but not the claimed composition with much higher concentrations of both PO₃ and PO₄.

Although Dolan and Fenn did study the effect of phosphate on the inhibition of infection by phosphorous acid, the two papers produced contradictory results with respect to the influence by phosphate on the fungicidal effects of phosphorous acid. For instance, Table 4 of Fenn (below) shows that increasing the phosphate concentration in the presence of phosphonic acid reduced the percent of inhibition. This was true of all seven genera of fungus as well as *Phytophthora cinnamomi*, where even the addition of 100X more phosphate

diminished the reported inhibition from 100% to 90%. Thus, Fenn actually teaches away from the present invention which teaches a much higher concentration of both PO_3 and PO_4 in the same composition.

TABLE 4. Percentage growth inhibition of various fungi on Ribeiro's synthetic agar medium (RMSM) containing 0.84 mM H_2PO_4 (69 $\mu\text{g}/\text{ml}$) at three phosphate concentrations

Fungus	Percentage inhibition of radial growth ^a at KH_2PO_4 concentrations (mM) of:		
	0.084	0.84	8.4
<i>Phytophthora cinnamomi</i> (Pc356)	100 a	93 a	90 a
<i>Pythium aphanidermatum</i>	53 b	56 b	31 b
<i>Rhizopus stolonifer</i>	52 b	30 c	0 c
<i>Fusarium oxysporum</i> f. sp. <i>apii</i>	42 b	5 d	1 c
<i>Verticillium dahliae</i>	49 b	0 d	1 c
<i>Schizophyllum commune</i>	38 b	0 e	2 c
<i>Rhizoctonia solani</i>	3 c	0 e	0 c

^aPercentage based on colony growth on identical medium without H_2PO_4 . Values are means of four or five replications. At a particular KH_2PO_4 concentration, values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Dolan presents data using similar concentrations where tomato seedlings were inoculated with *Phytophthora palmivora* to assess the effects of increasing phosphate content upon fungal infection rate. The relevant results are shown in Dolan's Table 4 below:

TABLE 4. Effect of 1 or 10 mM potassium phosphate levels on the percent inhibition of infection of tomato seedlings treated with phosphorous acid (H_3PO_3) or fosetyl-Na and inoculated with either the parental isolate of *Phytophthora palmivora* (PO376) or a mutant strain (L3) exhibiting high resistance to H_3PO_3 .

Treatment (PO_3 meq/L) ^x	Phosphate level (mM)	Percent inhibition of infection ^y			
		PO376		L3	
		H_3PO_3	Fosetyl-Na	H_3PO_3	Fosetyl-Na
0.85	0	57 d	6 g	17 f	0 c
2.43		100 a	36 e	59 c	0 c
6.10		100 a	100 a	71 b	2 c
0.85	1	72 c	19 f	39 e	3 c
2.43		100 a	42 d	48 d	4 c
6.10		100 a	99 a	73 b	3 c
0.85	10	87 b	69 c	45 de	39 b
2.43		100 a	91 b	75 b	45 a
6.10		100 a	100 a	82 a	45 a

^x Bare-rooted seedlings were placed in solutions of H_3PO_3 and of fosetyl-Na with 0, 1, or 10 mM potassium phosphate buffer and inoculated immediately with zoospores. Four days after inoculation, the stem of each seedling was plated in 0.5-cm segments on PARP medium to determine percent infection. Values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

^y Values for PO_3 meq/L were determined by dividing micrograms per milliliter by the conversion factors: 82 (H_3PO_3) or 132 (fosetyl-Na).

These results appear to controvert Fenn's Table 4, specifically, by showing that the addition of phosphate improves inhibition against *P. palmivora*. Dolan noted this discrepancy with the results obtained from the similar study by Fenn, concluding on page 977 only that more research is warranted:

The enhanced level of control of *P. palmivora* in vivo in the presence of increasing levels of phosphate was unexpected. It contradicted findings obtained with the interaction between *P. cinnamomi* and *Persea indica*, where phosphate was shown to reduce the efficacy of both compounds [here citing the work by Fenn and Coffey]. This indicates that phosphate influence on host and fungal metabolism may be an important factor affecting the efficacy of phosphonate fungicides. The relationship between phosphate concentration in tissues host parasite metabolism, and the mode of action of phosphonate fungicides could be complex. *There is need for additional research in this area to clarify the role of the host in these interactions* [emphasis added].

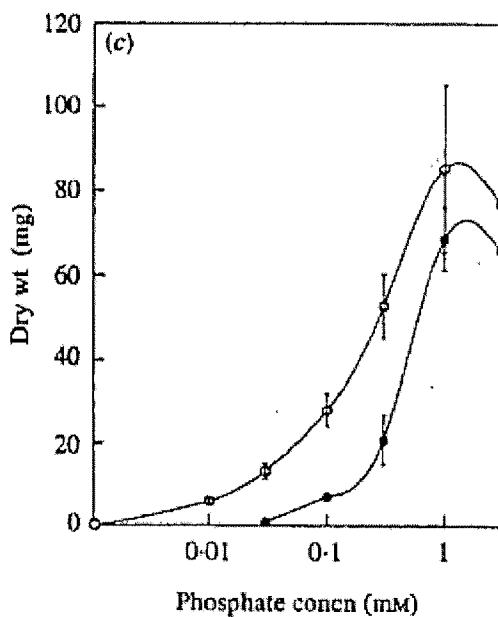
Page 977, Column 2, lines 1-10 of Dolan.

Thus, the Examiner's own references admit that they did not understand the phenomenon and could not produce consistent results, and that more research was warranted. At most, Dolan and Fenn disclosed that the relationship between phosphate and phosphite

concentrations and their effects on infection inhibition may be very complex. Therefore, there is no clear teaching in either Dolan, Fenn, or both that would motivate one of ordinary skill to modify the composition taught in these two references by increasing the concentration of both phosphate and phosphite in order to arrive at the composition presently claimed by Applicant.

Indeed, another reference of record, Griffith, which is not cited by the Examiner in the instant Office Action attempts to explain why Fenn and Dolan contradict one another. J. M. Griffith, M.D. Coffey, and B.R. Grant 1993, "Phosphonate inhibition as a function of phosphate concentration in isolates of *Phytophthora palmivora*," J. OF GENERAL MICROBIOL., 139: 2109-2116 (Griffith, Coffey & Grant). Griffith's results came from the same *P. palmivora* organism that was also the subject of the Dolan work.

In Griffith, *P. palmivora* was grown on medium that was enriched with phosphate at concentrations ranging up to a maximum of 1 mM. A "control" was performed for each phosphate concentration, and growth of these populations at each concentration were compared to growth on media that was also enriched with 1 mM phosphonate. These are extremely dilute concentrations of phosphate and phosphonate. Fig. 3 of Griffith shows that the relative inhibition effect which is caused by combining phosphonate with phosphate diminishes towards 1 mM. Griffith also discusses in Fig. 1(c) with regard to a mutant *P. palmivora* strain that is resistant to phosphonate. Fig. 1(c) is replicated below:



The Examiner can see from the above Fig. 1(c) that the observed inhibitory effect diminishes above 1 mM of phosphate concentration. Griffith also states in the discussion of Fig. 1(c) on page 2112 (discussing isolate P7228):

However, at higher levels of phosphonate (0.3 mM and above) phosphonate was less inhibitory to growth, and resistance to the effects of this anion was clearly demonstrated.

The overall trend as to the diminishing inhibition effect with increasing phosphate content is true with respect for all isolates in the Griffin study, which says on page 2113 that the upper limits for the observed effect were in the range of from 1 mM to 3 mM:

However, when P_i_e (phosphate content in the media) did not limit growth, at 1 mM and 3 mM, the P376 and P7228 strains accumulated more Pi (internal phosphate content in the cells) . . . than P113)

It will be appreciated that the P376 strain is one of the two mutant strains resistant to phosphonates that Dolan investigated, and that Griffith reports a much more thorough investigation. In Dolan, the levels of phosphate and phosphonate used were 10 mM and lower, which are also well below the concentrations that are now claimed.

Other work by Griffith shows that the metabolic interaction is more complex than one might otherwise imagine. The following Table is copied from J.M Griffith, R. H. Smillie, J.O.

Niere and B. R. Grant, 1989 Effect of phosphate on the toxicity of phosphonate in *Phytophthora palmivora*, ARCH. MICROBIOLOGIA 152:425-429

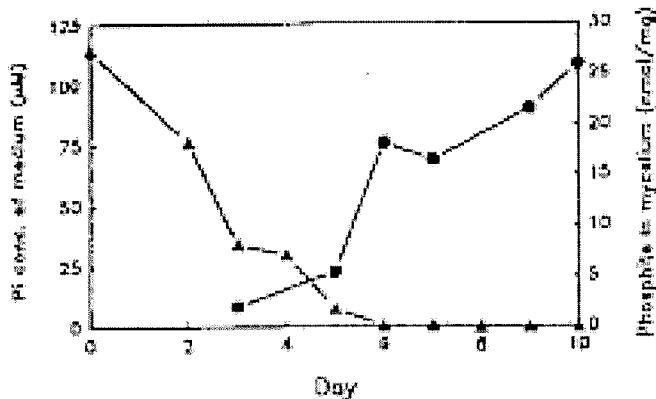


Fig. 1. The uptake of phosphate and the utilization of Pi by *Phytophthora palmivora* during growth in LPR medium containing 1 mM phosphate. Pi and phosphate concentrations were determined by ion chromatography as described in Methods. Pi in medium (▲—▲); phosphate in mycelium (●—●)

Griffith explains the significance of Fig. 1:

Analysis of the phosphite [phosphonate] content of the mycelium grown in LPR medium in the presence of 1 mM phosphite (the concentration used by Fenn and Coffey in 1984) showed that there was an abrupt increase in the level of phosphite entering the mycelium after Pi [phosphate] had been depleted from the medium at day 6 (Fig. 1).

This is shown above in Fig. 1 where the curve on the left hand side represents diminishing phosphate content in the growth medium, and the curve on the right hand side represents phosphonate that has entered the fungal cells of *P. palmivora*. At these concentrations, the phosphonate does not start to work until the phosphate is depleted. This explains, for example, why "[p]hosphates have also been considered to be a competitive inhibitor for phosphonate assimilation, thus inhibiting the ability of phosphonates to protect against fungus attack." U.S. 5,997,910, column 2, lines 57-60. But this is presented as a basis

in support of patentability where the art shows generally that phosphates should not be mixed with phosphonates to achieve an antifungal effect.

In summary, at the time of the present invention, the fungicidal or growth effects of relatively high concentrations of phosphate and phosphonate such as those claimed in the instant claims were not well understood. Indeed, Applicant has discovered that higher concentrations according to the claimed "effective amounts" produce an effect that differs in kind from what Fenn, Dolan and Griffith did. Thus, the claimed invention is not rendered obvious by Fenn and Dolan. Withdrawal of the 103 rejection is respectfully requested.

For the foregoing reasons, Applicant's attorney respectfully submits that the claims are worthy of allowance. Please charge the \$405 fee for RCE and \$230 fee for two-month extension of time to Deposit Account No. 12-0600. Applicant believes no additional fees are due, however, if any additional fee is deemed necessary in connection with this Response, please charge Deposit Account No. 12-0600.

Respectfully submitted



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